RESEARCH ARTICLE

Synthesis and Modification of Some New Progdrug Polymers Based on Ethylene Diamine Tetra Acetic Acid with Chitosan and Study Some of their Applications

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ABSTRACT

In this study, several medications with carboxylic groups (Diclofenac, Ciprofloxacin, Methyldopa) were used to make novel polymers for ethylene diamine tetra acetic acid (EDTA) and chitosan, which were then transformed to chloride pharmaceuticals by SOCl2. The preparation of a new variety of drug delivery systems (DDS) allows us to modify and significantly improve drug polymer's new therapeutic efficiency and safety (eliminates the toxic side effects). The fourier transform infrared spectroscopy (FTIR) and 1H-NMR spectra of the resultant compounds were used to describe them.

Keywords: Chitosan, EDTA, EDTA-chitosan Polymer, Progdrug Polymers.

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INTRODUCTION

Chitosan is a polymeric material and polysaccharide that is partially de acetylated from chitin, a crustacean protein that can be removed. N-acety[l] glucosamine and glucosamine units are connected by (1–4) glycosidic linkages to create it. Chitosan has a commercial benefit because of its biodegradability, chelating, adsorption power, good bio-compatibility and non-toxicity. Chitosan has a wide range of uses in the pharmaceutical and biotechnology industries, cosmetics, agriculture, and environmental engineering.[2] Chitosan is utilized in various pharmaceutical products, such as blood anticoagulants, controlled or release medication delivery systems. It can be dissolved in diluted organic and inorganic acids with pH values < chitosan's (pKa ~ 6.3).[3] Primary and secondary OH groups on C-2, C-3 and C-6 positions and NH2 groups are reactive functional groups in chitosan. The amino groups in chitosan are the most important determinants of their physical and chemical properties and structures, and they are linked to flocculation, biological roles, and chelation.[4] A polymer is a big molecule made up of several smaller molecules called monomers bound together.[5] Biopolymers, more specifically, are natural polymers produced by microorganisms, plants and animals. Naturally, generated biomass polymer is referred to as a "first class biobased polymer," whereas bio-engineered polymer is referred to a "second class biobased polymer."[6]

Because of its applications in medicinal and industrial domains, biodegradable polymers have sparked a lot of attention. Using biodegradable polymers derived from renewable resources can help to reduce environmental issues while also reducing the use of artificial polymers.[7] The degree of contact, molecular mass, and structure are all factors that influence the properties of biopolymers. The process of creating biopolymers with the desired properties is complicated and time-consuming. The biopolymer mixing method is used to control this problem, but this technique necessitates special attention to the mixture of biopolymers and their interactions.[8] Biopolymer-based delivery systems are important because of their wide range of applications, ease of use, and lack of toxicity.[9]

The DDS is the most beneficial application to human health interest and development field for biomedical substances. The DDS is defined as a formula that controls the period and rate of drug delivery and targets specific areas in the body. DDS is prepared to maintain drug levels throughout the treatment period.[10] Polymeric DDS are being studied for various uses to supplement current medical therapies. This drug delivery device is less sophisticated and smaller than conventional mechanistic pumps, allowing the drug to be stored as a dry powder in a polymer, as well as responding to changes in ambient conditions, e.g., pH, electric field,

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temperature, confirmed chemicals are search and light (visible or ultraviolet).[11] The rate of medication release in vivo and the optimal treatment concentration are regulated by drug delivery systems.[12] By using the metal ion-chelating mechanism, EDTA is an effective broad-spectrum inhibitor of metalloproteases, and it only requires a low concentration of 1–10 M. EDTA with free carboxyl groups, on the other hand, is scarcely soluble and frequently requires a pH more than 7 to achieve monomolecular dispersion, with two of the four carboxyl groups ionized.[13] Furthermore, due of substantial dilution and clearance during passage, a high dose is usually required when employed as an enzyme inhibitor for oral administration of proteins and peptides. Because of the potential interference with metal ion-dependent biological processes,[14] a safety issue may arise in this scenario, particularly for long-term use. The difficulties mentioned above can be partially solved by converting EDTA to chitosan via mucoadhesion. However, the synthesis procedure necessitates a complex pH adjustment, and the resulting conjugate is insoluble in acid medium due to the attachment of EDTA to chitosan's[15] main amino groups. In neutral and alkali liquids, the conjugate usually forms a clear gel but not a true solution.[16]

METHODS

Preparation of EDTA-Chitosan Conjugate (A)

Chitosan–EDTA conjugates were synthesized in a slightly modified way as described previously by Bernkop-Schnurch *et al*.[17] Thereby, 2 mmol of chitosan in dimethylsulfoxide (DMSO) with 1-mmol of EDTA and reflux the mixture 2–3 hours, then the product was washed by ethanol.

Preparation of Progdrug Polymers (A1-A3)

In the first step, convert the carboxylic drugs (Diclofenac, Ciprofloxacin, Methyldopa) to chloride drugs by reaction 1-mmol of drugs with 1-mmol thionyl chloride $SOCl_2$. Then 1-mmol. from polymer (A) refluxed (1-hour) with 4 mmol chloride drugs to produce polymers A1-A3 were washed in ethanol **(**Scheme 1) (Tables 1 and 2).

RESULTS AND DISCUSSION

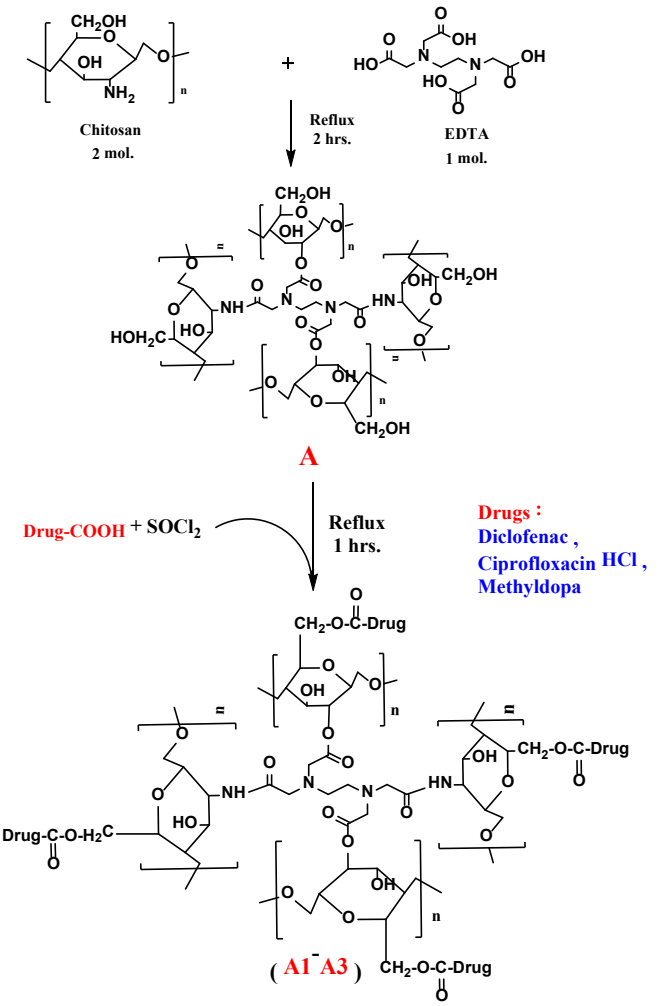
FTIR and ^{1}H-NMR

The FTIR analysis was carried out by means of KBr technique using Bruker spectrophotometer (400-4000 cm^{-1}). Likewise, nuclear magnetic resonance (^{1}H-NMR) analysis was carried out by dissolving the loaded drug in DMSO solvent using Varian Inova 500 MHz, spectrometer instrument.

FTIR spectrum showed a characteristic band for polymer A prepared from the reaction of chitosan with EDTA at 1605 cm^{-1} belonging to the amide C=O group and the disappearance of the C=O band at 1730 cm^{-1} belonging to the carboxylic C=O. The two NH_2 bundles of chitosan and the other prepared polymers (A1-A3) were diagnosed by the same methods. ^{1}H-NMR spectrum also showed a shift between 6 to 8 ppm due to the aromatic ring protons bound in drugs bound in polymers and their disappearance in others, as shown in the Figures 1 to 5.

Swelling Ratio and Drug Release

The polymers A1-A3 were investigated using acidic and basic functions with progressive hydrolysis at pH 1.2, 7, and 8 as pharmaceutical units of the drug-loaded polymer's hydrolysis (Table 3).



Scheme 1: A Schematic represents the preparation steps of progdrug (A1-A3)

Table 1: Drug release for progdrug (A1-A3) at pH = 1.2

Time (Day)	*A1*	*A2*	*A3*
1	0.191	0.091	0.129
2	0.212	0.130	0.159
3	0.231	0.145	0.172
4	0.251	0.172	0.191

Table 2: Drug release for progdrug (A1-A3) at pH = 7.0

Time (Day)	*A1*	*A2*	*A3*
1	0.321	0.260	0.267
2	0.332	0.266	0.274
3	0.342	0.271	0.283
4	0.365	0.281	0.298

The degree of polymer bonding is defined as the mechanical stress experienced during the bloating process because the higher the degree of entanglement, the greater the resistance to the bulge. According to the polymer and solvent molecules overlap, the polymer chain is not dissociated. Polymeric layers are inflated but do not disintegrate due to polymer saturation with the proper solvent.

Biological Activity

The biological activity of the polymer A1-A3 was investigated against both positive and negative bacteria (*Streptococci*) (*Pseudomonas*).

Anticancer Examination of the Progdrug A1-A3

Reagent preparation: MTT is soluble in ethanol (20 mg/mL), water (10 mg/mL), buffer salt solution, and culture medium (5 mg/mL). In the laboratory, dissolve 5 mg/mL of powder in PBS and gently vortex, then filter and store at -20°C for testing (Tables 4–6).

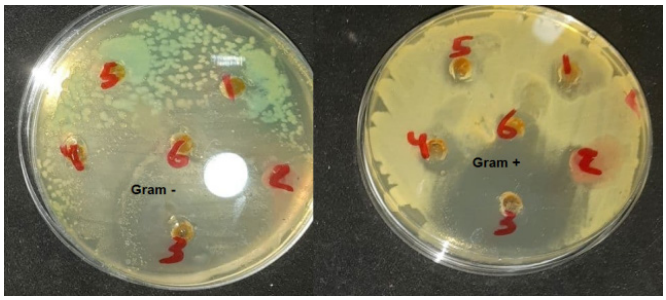


Figure 1: The effect of progdrug (A1-A3) on Streptococci and Pseudomonas bacteria for 24 hours.

Table 3: Drug release for progdrug (A1-A3) at pH = 8.0

Time (Day)	*A1*	*A2*	*A3*
1	1.043	0.483	0.802
2	1.089	0.534	0.825
3	1.122	0.601	0.853
4	1.145	0.639	0.881

Table 4: The swelling ratio% and Viscosity of progdrug (A1-A3)

Progdrug	*Swelling ratio %*	*Viscosity (dl/g)*
A1	12	0.77
A2	13	0.81
A3	9.4	0.65

Table 5: The biological activity of progdrug (A1-A3)

Progdrug	*Streptococci (Gram +)*	*Pseudomonas (Gram -)*
A1	19	13
A2	50	48
A3	12	10

Table 6: IC_{50} value for progdrug (A1-A3)

Progdrug	*IC_{50}*
A1	86.72
A2	388.51
A3	548.06

MTT Method Test

The American Type Culture Collection provided the MCF-7 (ATCC, Manassas, VA, USA) human breast cancer cell line, with 5% CO_2 in the air at 37°C; cells were grown and maintained in (DMEM; USA) Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (FBS; France) and 1% PSF (antibiotic antimycotic solution, USA). Cells were detached using 0.25% trypsin (Gibco, USA) and 0.1% EDTA (Merck, Germany) in phosphate-buffered saline at 37°C after attaining 75% confluence. The cells were then re-suspended in DMEM with 10% FBS and 1% PSF. Prior to the studies, cells were seeded at a density of 5000 cells per well in 96-well plates and incubated for 24 hours. The cells were rinsed in PBS (phosphate Buffered Saline, pH

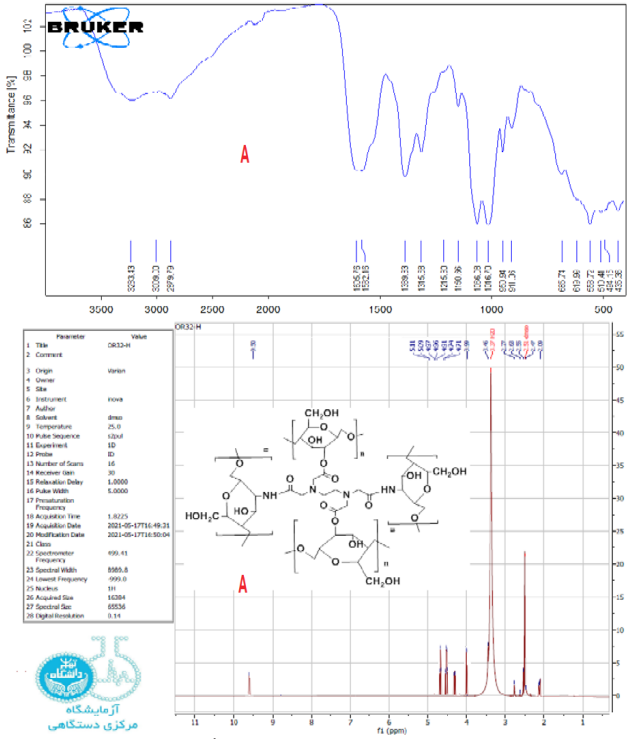


Figure 2: The FTIR and ^{1}HNMR spectrum of EDTA-chitosan polymer A

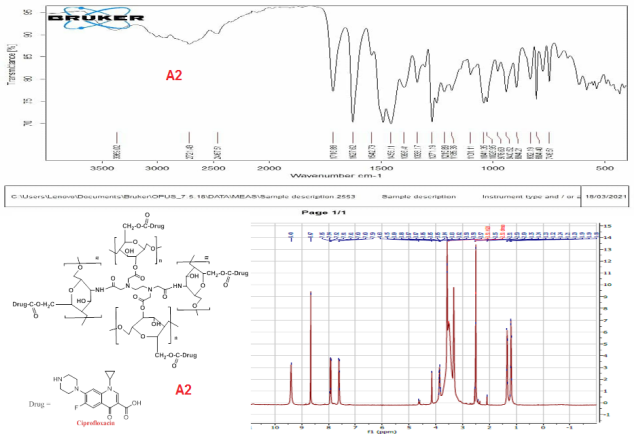


Figure 3: The FTIR and 1HNMR spectrum of EDTA-chitosan progdrug polymer A_1

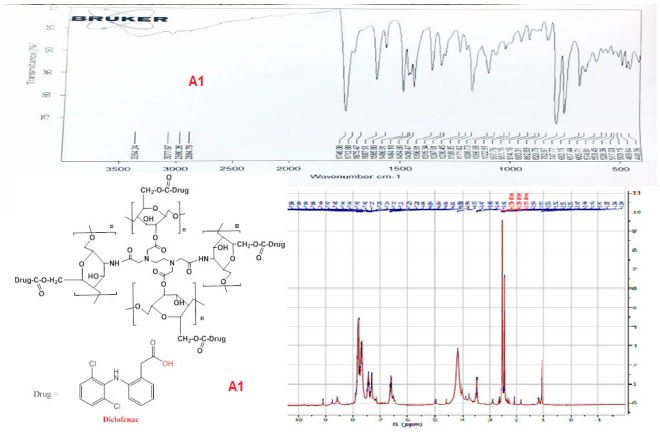


Figure 4: The FTIR and 1HNMR spectrum of EDTA-chitosan progdrug polymer A2

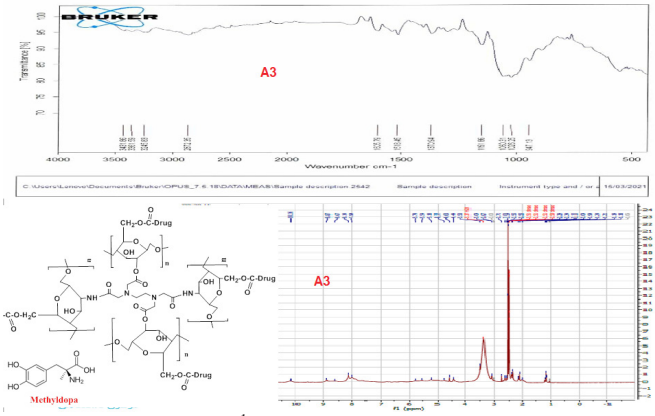


Figure 5: The FT-IR and ^{1}HNMR spectrum of EDTA-chitosan polymer (A) and progdrug (A1-A3)

7.4) before being cultured for 72 hours in fresh media with various concentrations of samples (1000, 500, 250, 125, 62.5, 31.25, 0, 0 g/mL). The 3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) dye reduction test was used to determine cell viability. The cytotoxic effect of the materials at varying concentrations was determined using MTT. MTT (5 mg/mL in PBS) was added to each well after 72 hours of incubation (37°C, 5% CO_2 in a humid atmosphere). The plate was then incubated for another 4 hours at 37°C. The formazan was dissolved in 100 mL of DMSO with moderate shaking at 37°C, and absorbance was measured with an ELISA reader at 570 nm. The results were calculated as the average of three separate studies. The concentrations of substances that reduced cell viability by 50% (i.e., IC50 values) were computed.[18-21]

CONCLUSIONS

New drug polymers were synthesized by combining chitosan with EDTA and then combining it with carboxylic medicines. The FTIR and 1H-NMR spectra of all of these polymers revealed distinctive bands indicating the creation of the intended target molecules from the starting material. All of the produced polymers' physical characteristics were investigated. The quick-release of polymers that had been prepared was investigated. Hydrolysis was carried out using acid and base functions at pH 1.2, pH 7, and pH 8. The biological activity of the following polymers was examined against positive and negative bacteria using an Ostwald viscometer and it was discovered that the progdrug A3 had stronger biological activity against (+) positive bacteria and (-) negative bacteria.

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